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COMMUNICATION

Cystine oligomers successfully attached to peptide cysteine-rich fibrils

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Supporting Information

Experimental

Transmission electron microscopy (TEM)

TEM images were recorded with a Philips C20 transmission electron microscope. To prepare TEM samples, small aliquots of fibril solutions were deposited onto freshly glow-discharged copper grids covered by a thin carbon layer. A droplet of 3 μL of the fibril solution was deposited on the carbon film for absorption for 1 minute—the excess of fluid was blotted away.

Grids were rinsed by applying some droplets of distilled water, followed by blotting. All samples for TEM measurements were negative stained with a solution 2% phosphotungstic acid (PTA) adjusted to pH 7.4.

Peptide gold nanoparticles (Au NPs) labelling

Gold Nanoparticles stabilized suspension in citrate buffer was purchased from Aldrich (diameter nearly 10 nm, 5.5×10^{12} part./mL). Here, unlike the TEM sample-preparation procedure described above, after having performed the peptide deposition and after rinsing, the grid was rapidly (i.e., without drying) positioned upside-down onto a 20 μL drop of Au NPs solution 5×10^{10} part./mL. The grid was placed in such a way as to avoid any kind of precipitates on its surface. This in-situ reaction was let to proceed for several minutes (typically between 1 and 10) depending on the specific experiment. Then, after rinsing, the grid was put on a 15 μL drop of the same PTA solution formerly employed. Within this procedure, grids were positioned on the drops and then transferred between the drops by means of a pair of tweezers. At each transfer, most of the former solution was removed by rapidly (but gently) touching the grid to a filter paper.

Synchrotron radiation circular dichroism (SR-CD)

SR-CD spectra were collected on the AU-UV beam line on the ASTRID2 storage ring (ISA, Aarhus University, Denmark). Similar to the CD experiment previously described on the CD1 beam line on ASTRID, light from the AU-UV beam line was polarised with a MgF_2 Rochon polarizer (B-Halle GmbH, Berlin) and a photo elastic modulator (Hinds, USA) produced alternating left and right handed circular polarized light. The light was then passed though the

sample, with concentrations of 1 mg/mL, and was detected by a photo multiplier tube (Type 9406B, ETL, UK). Spectra of water were recorded for baseline subtraction.

Samples were measured in a 0.1 mm path-length Suprasil cell (Hellma GmbH). Sample and baseline spectra (1 nm steps size and 2 second dwell time) were each collected in triplicates, averaged and slightly smoothed with a Savitzky-Golay filter using a purpose made Excel template. For Dichroweb, the data analysis utilised was the CDSSTR program with reference set SP175 (Optimised for 175-240 nm) and no scaling factor (i.e. the scaling factor is set equal to 1.0).

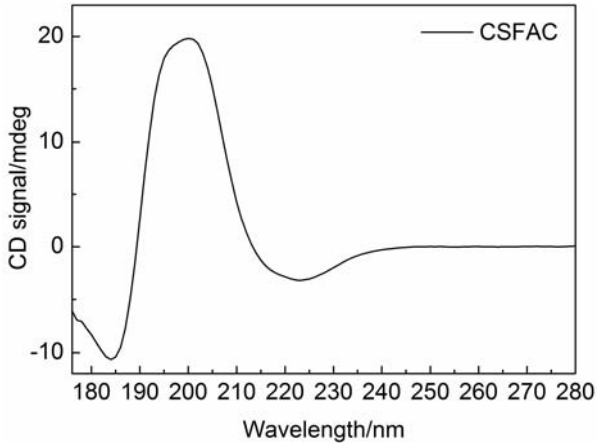

Fig. S1 Synchrotron radiation circular dichroism (SR-CD) spectrum of CSFAC fibrils. The characteristic positive peak at 200 nm and the negative peak at 220 are indicative of a β-sheet major contribution to the secondary structure of the cysteine-rich fibrils.

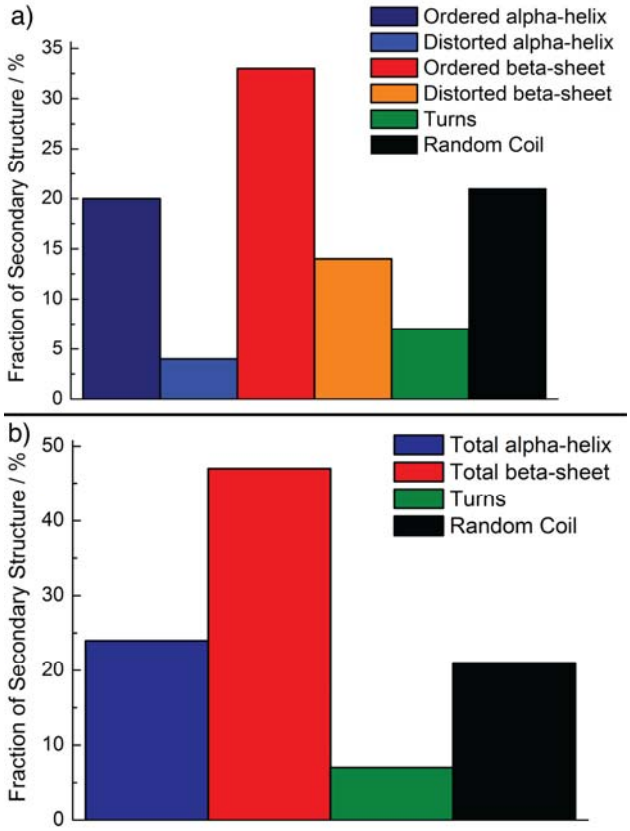

Fig. S2 Histograms presenting the computed values of secondary structure fractions performed with Dichroweb (see main text for details). a) Fractions of individual contribution—ordered structures prevail on the distorted ones; b) Overall comparison of α-helices and β-sheets—the latter dominates and account for almost 50% of the total.

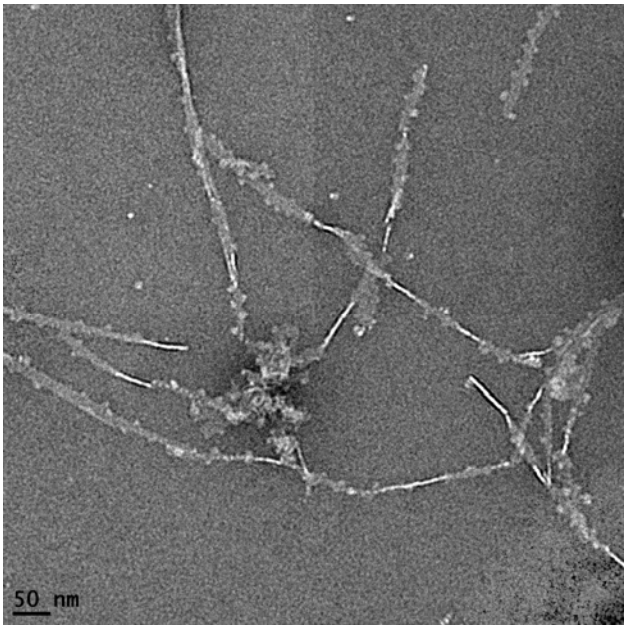

Fig. S3 Negative stained TEM image of CSFAC fibrils decorated with cystine oligomers